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16. ABSTRACT

Pathological changes in the central nervous system in two strains of mice (BALB/c and C57BL/6) and two strains of rats as a result of trimethyltin (TMT) intoxication were compared. Both strains of mice were administered with trimethyltin chloride at a dosage of 3.0 mg TMT-Cl/kg b.w. while both strains of rats were exposed to 7.5mg TMT-Cl/kg b.w. Animals were sacrified at the time of development of observable neurological signs (tremor, aggression): 2 days for both strains of mice, 3 days for Long Evans (LE) rats, and 5 days for Sprague Dawley (SD) rats. It was found that there were both species and strain differences in TMT toxicity. Despite being exposed to a lower dose of TMT and for a shorter duration of time, mice showed more prominent neurological signs and hippocampal lesions than rats. Among the two strains of rats studied, LE rats were more sensitive than SD rats to TMT toxicity. The regional sensitivity of the CNS between mice and rats was also different, with mice showing most lesions in the hippocampal fascia dentata and rats showing more prominent neuronal damages in the olfactory cortices and hippocampal Ammon's horn. Our present investigation provides the first species/strain comparison on lesion development as a result of TMT intoxication.

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Species and Strain Comparison of Acute Neurotoxic Effects of Trimethyltin in Mice and Rats[1]

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CHANG, L. W., G. R. WENGER, D. E. McMILLAN AND R. S. DYER. *Species and strain comparison of acute neurotoxic effects of trimethyltin in mice and rats.* NEUROBEHAV TOXICOL TERATOL 5(3) 337–350, 1983.—Pathological changes in the central nervous system in two strains of mice (BALB/c and C57BL/6) and two strains of rats (Long Evans and Sprague Dawley) as a result of trimethyltin (TMT) intoxication were compared. Both strains of mice were administered with trimethyltin chloride at a dosage of 3.0 mg TMT-Cl/kg b.w. while both strains of rats were exposed to 7.5 mg TMT-Cl/kg b.w. Animals were sacrificed at the time of development of observable neurological signs (tremor, aggression): 2 days for both strains of mice, 3 days for Long Evans (LE) rats, and 5 days for Sprague Dawley (SD) rats. It was found that there were both species and strain differences in TMT toxicity. Despite being exposed to a lower dose of TMT and for a shorter duration of time, mice showed more prominent neurological signs and hippocampal lesions than rats. Among the two strains of rats studied, LE rats were more sensitive than SD rats to TMT toxicity. The regional sensitivity of the CNS between mice and rats was also different, with mice showing most lesions in the hippocampal fascia dentata and rats showing more prominent neuronal damages in the olfactory cortices and hippocampal Ammon's horn. Our present investigation provides the first species/strain comparison on lesion development as a result of TMT intoxication.

Strain differences Trimethyltin Neurological effects

INDUCTION of overt neurological and behavioral changes in rodents, including aggression, hyperirritability, tremor, spontaneous seizures, hyperreactivity, and changes in schedule-controlled behavior by trimethyltin (TMT) compounds are well documented [1–3, 11, 13]. In rats, such behavioral changes have been referred to as "the trimethyltin syndrome" [11].

Neuropathological studies with rats have revealed extensive lesion development in the limbic system, including the Ammon's horn (pyramidal neurons), fascia dentata, pyriform cortex, entorhinal cortex, and amygdaloid nucleus [1, 2, 9, 10]. Recent investigations have indicated that mice are much more sensitive to TMT toxicity, producing rapid lesions in the limbic system [5,6] and developing significant pathological changes in "extra-limbic" neurons such as those in the brain stem [4] and spinal cord [7,8].

Since some laboratories have worked with rats and others with mice, it is not clear if there are species differences in the neuropathology produced by TMT. If some qualitative and quantitative differences in neuropathology exist between rodent species and strains, in-depth analysis of those differences may lead to an understanding of the mechanisms by which the toxic changes occur. Furthermore, data are beginning to accumulate which suggest different behavioral effects of TMT on rats and mice [12,13]. A comparative analysis of the morphological consequences of TMT exposure may help to elucidate these species differences. As a first step toward such analyses, the present investigation was intended to establish a framework for comparative studies on TMT-induced lesion development in rats and mice.

METHOD

Two strains of mice, BALB/c and C57BL/6 (C57), and two strains of rats, Long Evans (LE) and Sprague Dawley (SD), in groups of six were used in this experiment. All animals used were young (60–90 day old) adult males. Trimethyltin chloride (TMT-Cl) was dissolved in a solution of physiologic saline and administered to mice (both strains) at a dosage of 3.0 mg TMT-Cl/kg b.w. and to rats (both strains) at a dosage of 7.5 mg TMT-Cl/kg b.w. Because of the steep dose-effect function for TMT [11], it is important to note that these dosages are here expressed as the total salt.

[1]The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement numbers R 809452-01 and R 809360-01 to Drs. L. W. Chang and D. E. McMillan. It has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Equivalent dosages of TMT as base would be 2.46 mg/kg for mice and 6.17 mg/kg for rats. Control animals (six per strain) were treated with the saline vehicle. Dose response studies for the SD and LE rats and for the BALB/c mice had been established in previous studies [2, 11, 13]. The doses selected for our present study represent sub-LD_{50} doses which were found to be effective in the induction of neurological signs and lesions in these animals.

Animals were sacrificed at the time when observable early neurological signs or behavioral changes (tremor and aggression) developed: 2 days for the mice (both strains), 3 days for LE rats, and 5 days for SD rats. All animals were deeply anesthetized and sacrificed via intracardial perfusion with saline solution followed by 2.5% buffered glutaraldehyde. After perfusion, brains and spinal cords were removed carefully and further fixed in 10% buffered formalin for at least 48 hours. Tissue specimens were then dehydrated with graded ethanols, cleared with xylene, and embedded in Paraplast. Tissue sections were made (6–8 μm thick) at both cross-sectional and parasagittal planes. All sections were stained with hematoxylin-eosin (H and E) for light microscopic examination. For consistency purposes, tissues were compared at approximately the same levels of sectioning (Diagram 1). Lesions (cell generation and cell death) were rated between − to + + + + in reflection of extent of pathological involvement. All ratings were done under blind conditions by the same investigator throughout the experiment.

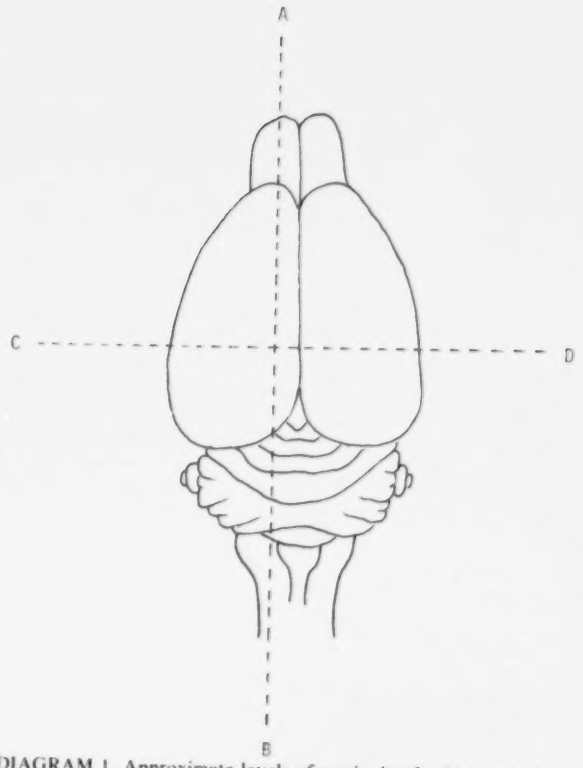

DIAGRAM 1. Approximate levels of sectioning for histopathology comparison. A–B, parasagittal section through midline of olfactory bulb; C–D, coronal section through the infundibulum of the hypophysis at the base of the brain.

RESULTS

Neuropathology in Mice

Forty-eight hours after TMT exposure, whole body tremor developed in both strains of mice. Tremor was especially severe in the C57 mice. Histopathological examination revealed very similar distribution of lesions in both strains of animals involving the hippocampal pyramidal neurons, fascia dentata, pyriform/entorhinal cortices, olfactory tubercle, olfactory nuclei, spinal cord, and brain stem. No remarkable pathology was observed in other areas of the cerebral cortex and cerebellum at this time of intoxication.

The most extensive damage was found among the granule cells of the fascia dentata (Figs. 1 and 2). Many neurons became necrotic acquiring pyknotic nuclei and highly eosinophilic cytoplasm. It was also apparent that the fascia dentata of the BALB/c animals was even more sensitive to TMT-toxicity than that of the C57 mice, showing extensive necrosis and spongiosis (vacuolation) (Fig. 1).

Damage to the hippocampal pyramidal neurons was present but unremarkable in both strains involving only isolated vacuolar changes in some neurons of the $CA_{1,2}$ and CA_{3c} areas (Figs. 3 and 4). Neurons in the large pyramidal cells of $CA_{3a,b}$ area were mostly spared.

Scattered necrosis of the small cortical neurons was also found in the pyriform and entorhinal cortices and along the olfactory tubercle of both strains (Figs. 5 and 6). Neuronal necrosis and vacuolation were observed in the nerve cells of the olfactory nucleus (Figs. 7 and 8), especially in the BALB/c mice.

Degenerative changes in brain stem neurons and in spinal cord motoneurons were prominent in both strains of mice. Chromatolytic and vacuolar changes characterized the pathological alterations in these nerve cells (Figs. 9 and 10). In the brain stem, the mesencephalic trigeminal nuclei and the raphe nuclei were most severely affected. However, scattered neuronal degeneration was also present in other parts of the brain stem. In the spinal cord, both the medial and lateral motor nuclei were involved.

Neuropathology in Rats

The overall overt and pathological effects of TMT on rats were much milder and slower than those on mice. Both tremor and aggression were also observed in the LE and SD animals after 3 and 5 days of TMT intoxication. However, the tremors in rats were much milder than those displayed by mice.

Histopathological examination revealed that lesions were more extensive in the nervous system of LE rats than in SD rats. In the hippocampus of LE rats, neuronal swelling and necrosis were found in the fascia dentata and CA_{3c} areas (Figs. 11 and 12). Scattered neuronal death and vacuolation were also observed in the $CA_{1,2}$ area at this time (Fig. 13). The histopathology in $CA_{3a,b}$ neurons was not remarkable at this time. In comparison, SD rats showed only very minor changes in the fascia dentata and CA_{3c} neurons (Figs. 14 and 15).

While hippocampal neurons were less involved in rats than mice, pathological lesions in the olfactory tubercle and pyriform/entorhinal cortices were more prominent in rats (both strains) than in mice, showing significant neuronal loss in these areas (Figs. 16, 17 and 18). Brain stem lesions, particularly involvement of the mesencephalic trigeminal and

FIG. 1. BALB/c mouse, 3.0 mg/kg TMT-Cl, 48 hours. Extensive necrosis and vacuolation of the granule cells in the fascia dentata were observed. The extensive vacuolar change gave the tissue a spongiotic appearance. (×250)

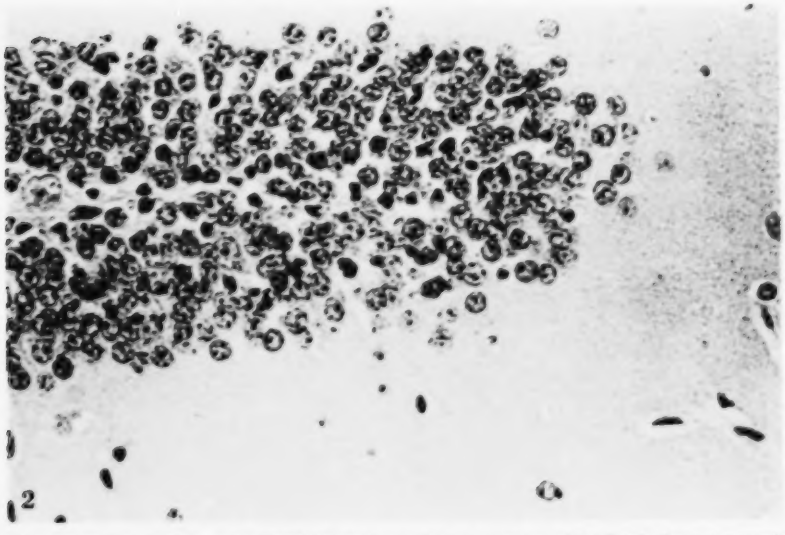

FIG. 2. C57 mouse, 3.0 mg/kg TMT-Cl, 48 hours. Large numbers of the fascia dentata granule cells appeared to be necrotic with dense-pyknotic nuclei and eosinophilic cytoplasm. (×250)

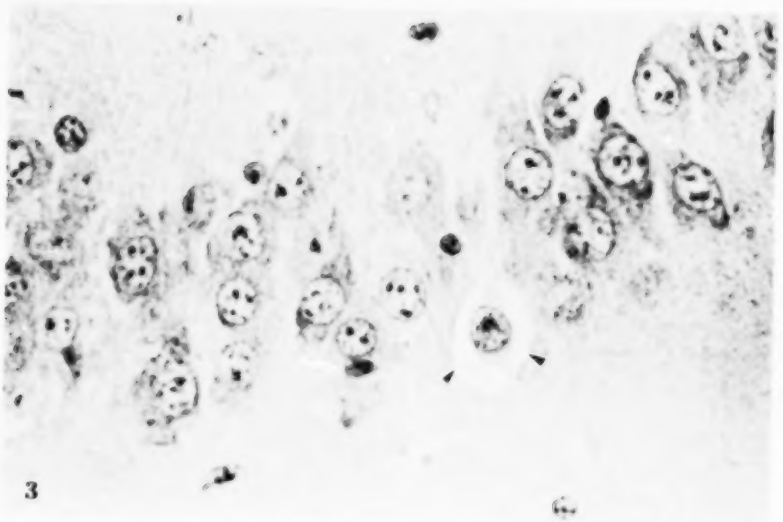

FIG. 3. BALB/c mouse. 3.0 mg/kg TMT-Cl. 48 hours. Ammon's horn. Despite the extensive damage in the fascia dentata granule cells, only isolated pyramidal neurons showed vacuolar changes (→) in the $CA_{1,2}$ area. (×450)

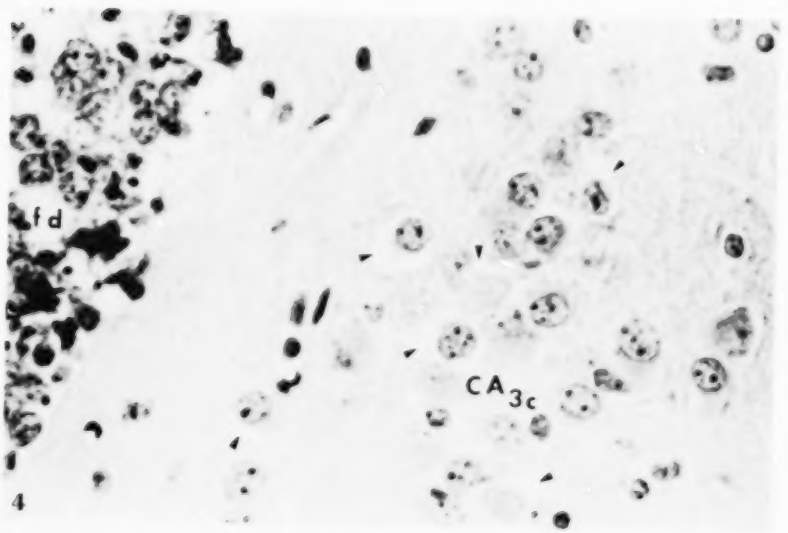

FIG. 4. BALB/c mouse. 3.0 mg/kg TMT-Cl. 48 hours. hippocampus. While extensive neuronal necrosis and vacuolation were observed in the fascia dentata (f.d.), only vacuolar changes (→) were observed in the pyramidal neurons in the CA_{3c} area. (×450)

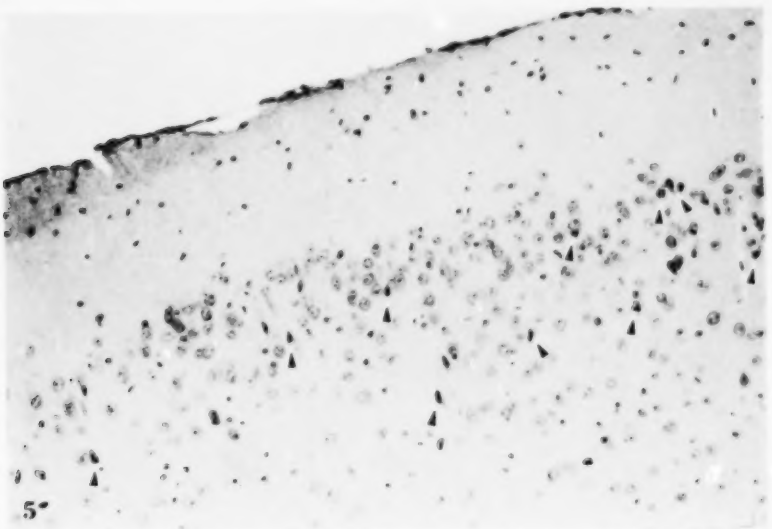

FIG. 5. BALB/c mouse, 3.0 mg/kg TMT-Cl, 48 hours, pyriform cortex. Isolated neuronal necrosis (→) also occurred in the pyriform and entorhinal cortices. (×100)

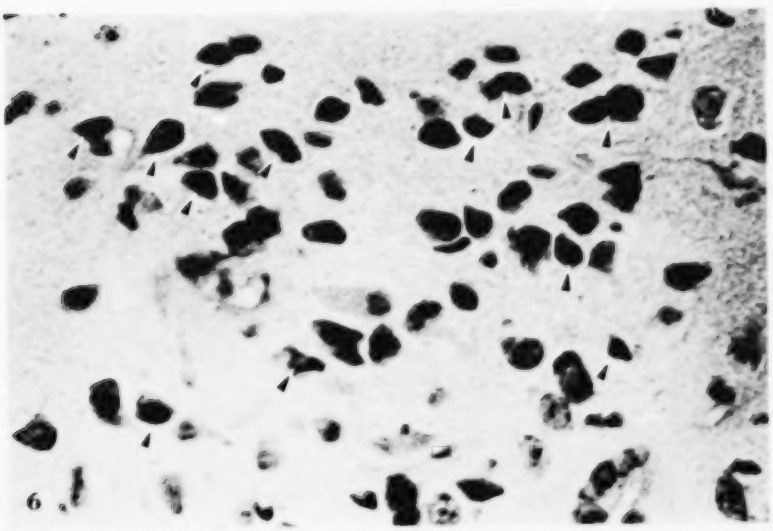

FIG. 6. C57 mouse, 3.0 mg/kg TMT-Cl, 48 hours, olfactory tubercle. Neuronal swelling and necrosis (→) were also evident in this area of the cortex. (×450)

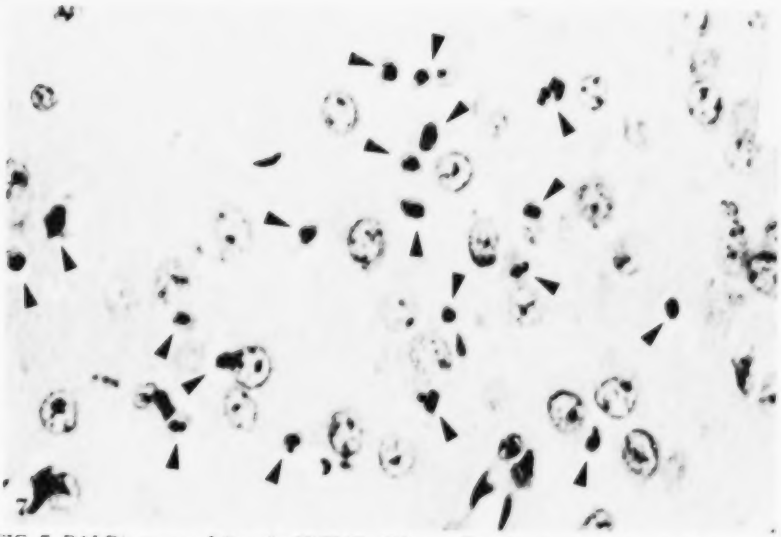

FIG. 7. BALB/c mouse, 3.0 mg/kg TMT-Cl, 48 hours. Prominent neuronal necrosis (→) was found in the medial anterior olfactory nucleus. (×450)

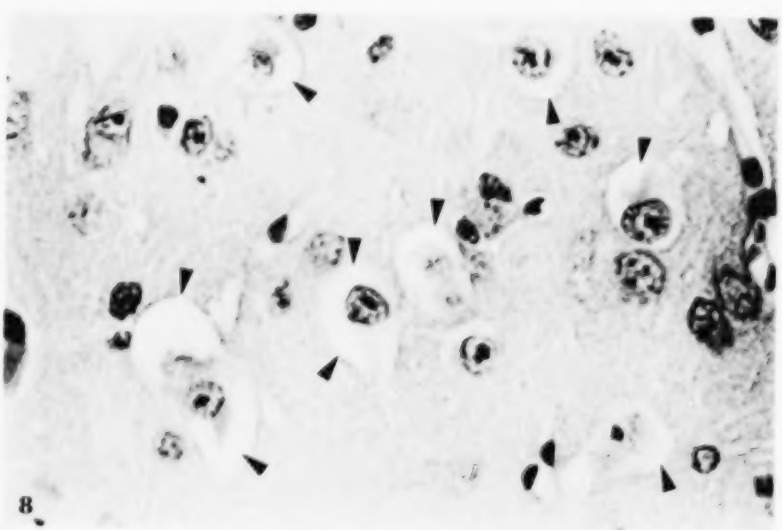

FIG. 8. BALB/c mouse, 3.0 mg/kg TMT-Cl, 48 hours, anterior olfactory nucleus. Vacuolar changes (→) were observed in this group of neurons. (×450)

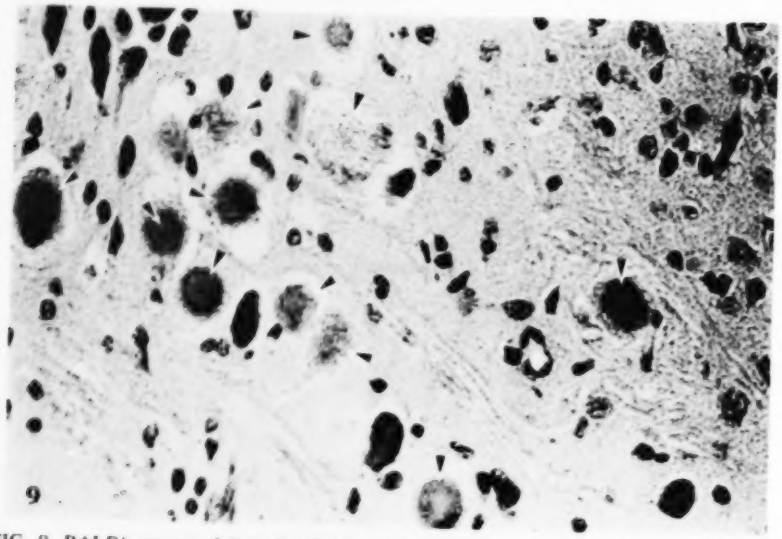

FIG. 9. BALB/c mouse, 3.0 mg/kg TMT-Cl, 48 hours, mesencephalic trigeminal nucleus. Marked chromatolytic and vacuolar changes (→) of the large brain stem neurons, especially those in the mesencephalic trigeminal and raphe nuclei, were observed. (×450)

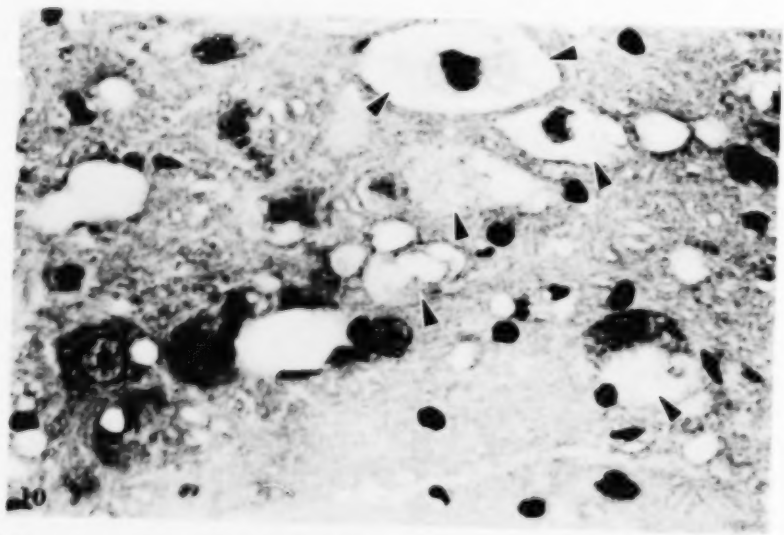

FIG. 10. BALB/c mouse, 3.0 mg/kg TMT-Cl, 48 hours, spinal cord. Extensive chromatolytic and vacuolar degeneration of many spinal motoneurons (→) were evident. These changes were found in both strains of mice studied but not in the rats. (×450)

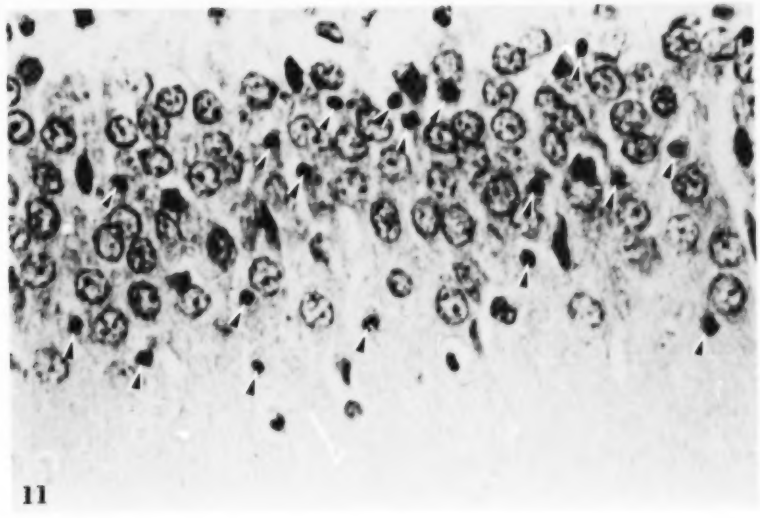

FIG. 11. LE rat, 7.5 mg/kg TMT-Cl, 3 days, fascia dentata. Significant neuronal necrosis (→) was observed in the granule neurons. (×450)

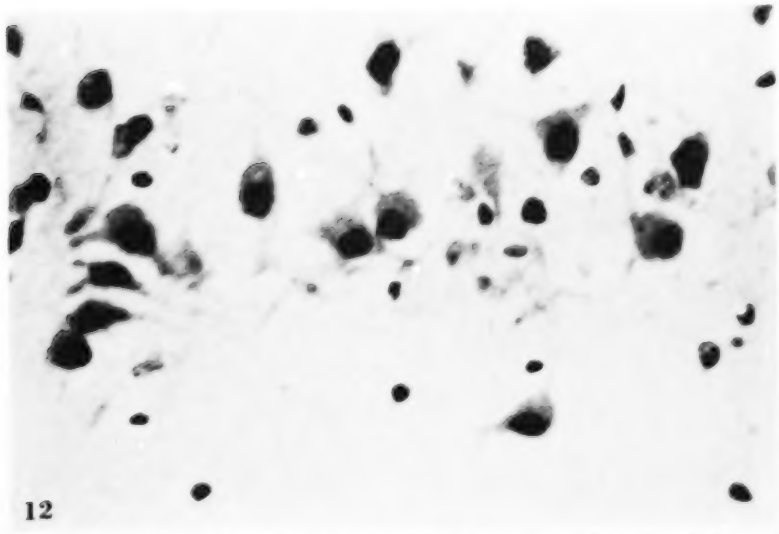

FIG. 12. LE rat, 7.5 mg/kg TMT-Cl, 3 days, Ammon's horn. Prominent neuronal changes, including swelling and necrosis, were observed in the CA_3 pyramidal neurons. (×450)

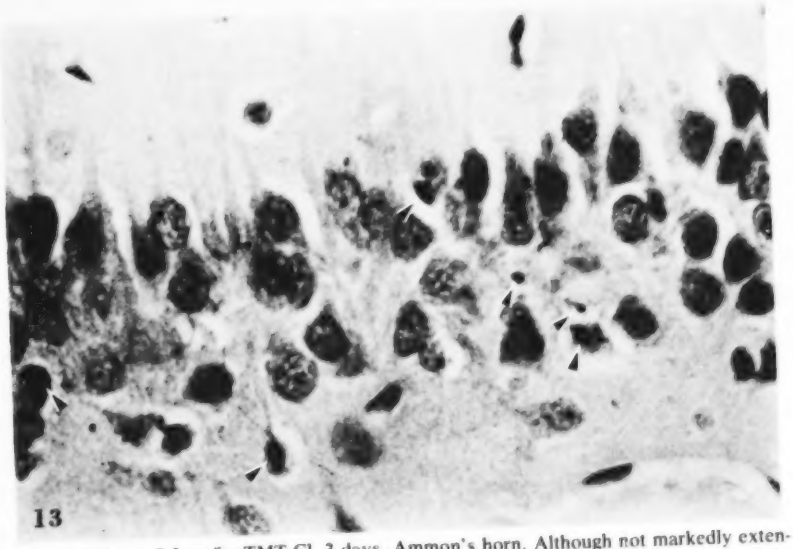

FIG. 13. LE rat, 7.5 mg/kg TMT-Cl, 3 days. Ammon's horn. Although not markedly extensive, scattered neuronal necrosis (→) was readily found among the pyramidal neurons in the $CA_{1,2}$ area. (×450)

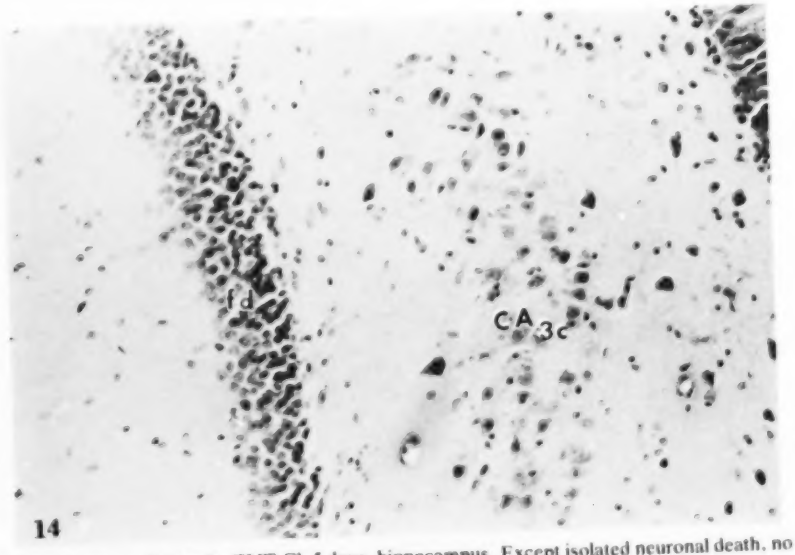

FIG. 14. SD rat, 7.5 mg/kg TMT-Cl, 5 days, hippocampus. Except isolated neuronal death, no significant damage was observed in the fascia dentata (f.d.). (×250)

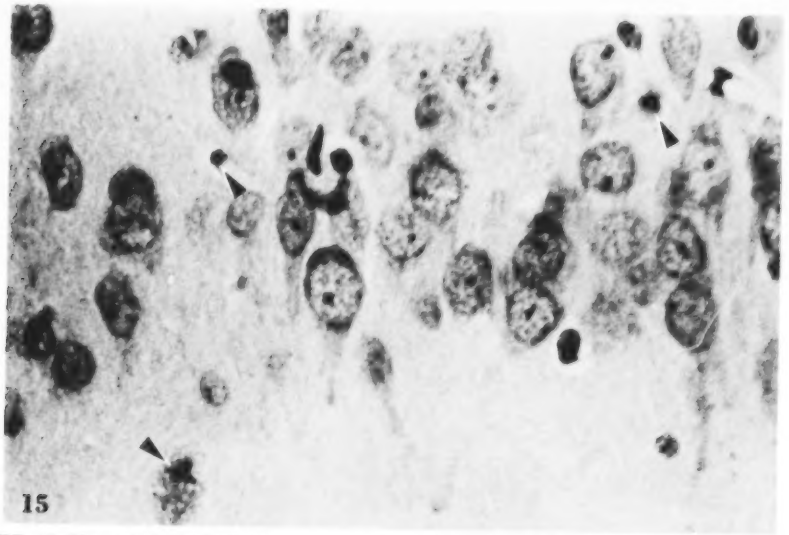

FIG. 15. SD rat, 7.5 mg/kg TMT-Cl, 5 days, Ammon's horn. While no damage occurred in the cells in $CA_{1,2}$ and $CA_{3a,b}$ areas, isolated neuronal death (→) could be found in the CA_{3c} neurons. (×450)

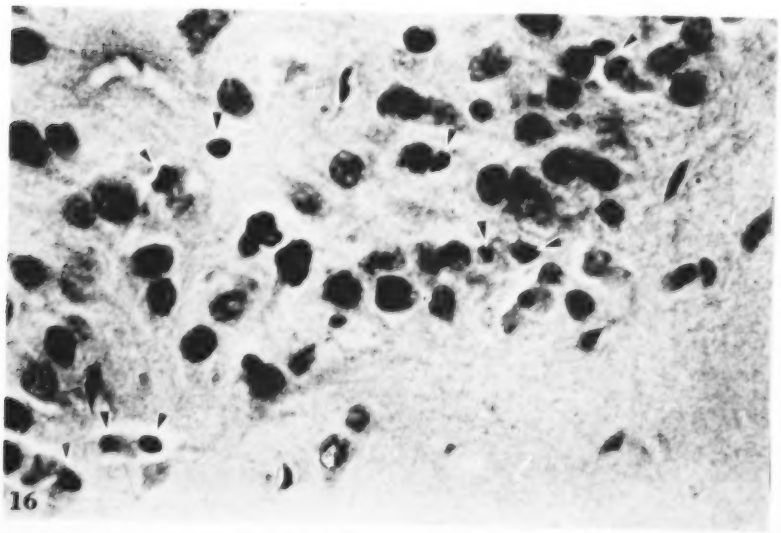

FIG. 16. LE rat, 7.5 mg/kg TMT-Cl, 3 days, olfactory tubercle. Neuronal swelling and necrosis (→) were found in this area of the cortex. (×450)

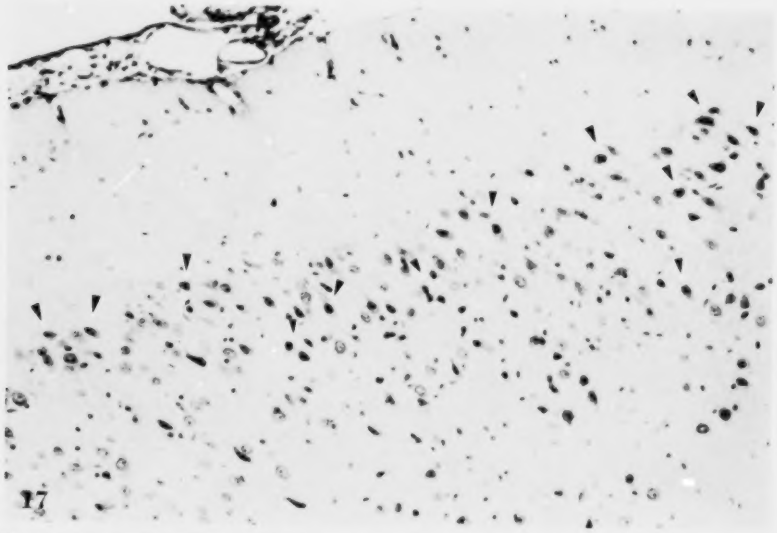

FIG. 17. LE rat, 7.5 mg/kg TMT-Cl, 3 days, pyriform cortex. Marked neuronal swelling and necrosis (→) were observed among the nerve cells of this cortex. (×250)

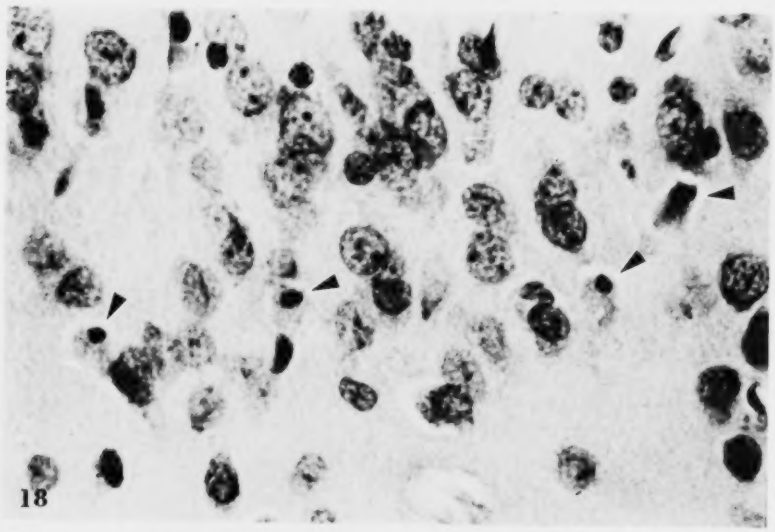

FIG. 18. SD rat, 7.5 mg/kg TMT-Cl, 5 days, pyriform cortex. Necrosis of the pyriform cortical neurons (→). (×450)

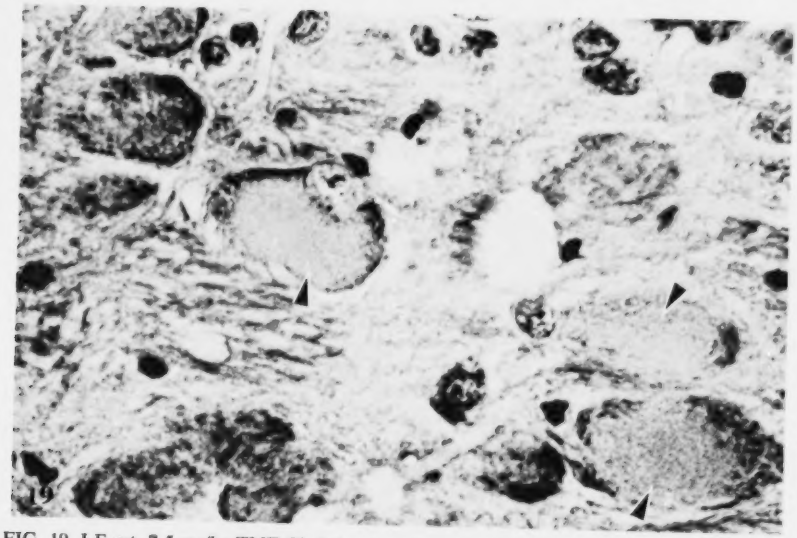

FIG. 19. LE rat, 7.5 mg/kg TMT-Cl, 3 days, mesencephalic trigeminal nucleus of brain stem. Marked chromatolytic change (→) was observed in these neurons. (×450)

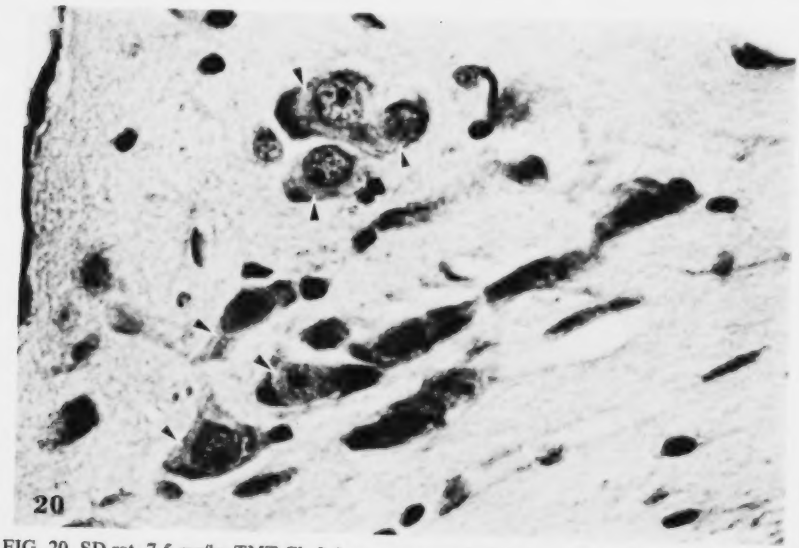

FIG. 20. SD rat, 7.5 mg/kg TMT-Cl, 5 days, mesencephalic trigeminal nucleus of brain stem. Although much milder than those observed in LE rats, chromatolytic change of these neurons (→) was also evident in the SD rats. (×450)

raphe nuclei, also occurred in both strains of rats. However, in rats these lesions were confined to chromatolytic changes (Figs. 19 and 20) without the vacuolar degeneration as those observed in mice. No histopathological changes were observed in the spinal motoneurons of rats under the present experimental conditions.

The extent of lesion development and distribution were quite consistent among animals in any given strain of rats or mice. For comparative purposes, the extent of pathological involvement in each strain of animals studied was estimated on a scale of − to ++++ and is summarized in Table 1.

DISCUSSION

The purpose of this report is to compare the extent of morphological lesion development in different species and strains of rodents (mice and rats) under the influence of TMT. Since details of morphological changes and pathology related to these species and strains as a result of TMT-intoxication has been described in previous publications [1–6, 9, 10], only general and relative pathological changes are presented in this report. For more detailed pathological assessment of changes in any specific area of the brain of a

TABLE 1

COMPARATIVE TMT-INDUCED LESIONS IN RATS AND MICE*

	Hippocampus							
	$CA_{1,2}$	$CA_{3a,b}$	CA_{3c}	f.d.	o.t.	p.c./e.c.	s.c.	b.s.
BALB/c (3.0 mg/kg, 2 d)	−/±	−	−/±	+++/++++	±	±	++	+/++
C57 (3.0 mg/kg, 2 d)	−/±	−	−/±	++/+++	±	±	++/+++	+/++
LE (7.5 mg/kg, 3 d)	±	−/±	+	±/+	+	+/++	−	+
SD (7.5 mg/kg, 5 d)	−	−	±/+	±	±/+	+	−	±

*All ratings were based on parasagittal sections of the dorsal hippocampus. All comparisons were made on approximately the same levels of sectioning (see Diagram 1). Spinal cords were examined at levels L_1-S_1. Extent and severity of lesions were rated from − to ++++ with − being no observable lesion and ++++ being very extensive involvement. (f.d., fascia dentata; o.t., olfactory tubercle; p.c./e.c., pyriform/entorhinal cortices; s.c., spinal cord; b.s., brain stem.)

particular strain of animal, readers should consult previous publications [3–9].

Under the present experimental design (single high toxic dose and short duration), it is evident that there are both species and strain differences in TMT toxicity. Furthermore, the species differences appear greater than the strain differences. Despite being exposed to a relatively lower dose of TMT for a shorter period of time, both strains of mice used in our investigation showed more severe symptomatology and overall lesions than those in rats. Although the difference between the two strains of mice (BALB/c and C57BL/6) was not very remarkable, there was a significant difference between the two strains of rats (LE and SD) studied. It is apparent that the LE rats were more sensitive than the SD rats, showing much more damage of the hippocampal neurons in a shorter period of time (3 days vs. 5 days).

The general lesion distribution or regional sensitivity between mice and rats was also different. For mice, the order of neuronal vulnerability to TMT appears to be: granule cells of fascia dentata > spinal cord motoneurons > brain stem neurons > olfactory cortical neurons (pyriform/entorhinal cortices and olfactory tubercle) > Ammon's horn pyramidal neurons. For rats, the equivalent comparison is: olfactory cortical neurons > Ammon's horn pyramidal neurons (particularly CA_{3c}) ≥ granule cells of fascia dentata > brain stem neurons. No spinal cord motoneuron damage was observed in rats. Reasons for these species/strain differences are still not known. It must be emphasized that our present findings reflect only acute toxic conditions (single high dose, short-term) in mice and rats. As chronic exposure (multiple low dose, long-term) may stimulate different toxic responses and reactions, the distribution and extent of neuronal damage may also vary.

The present study represents the first investigation, based on neuropathological criteria, of species/strain differences in TMT toxicity. There is no doubt that the experimental design (single acute exposure and sacrifice at time of symptomatology development) offers a limited scope for comparison. However, the study has provided the first morphological evidence of species and strain differences in lesion development after TMT. This information should serve as an important cornerstone for future investigations on both structural and functional (physiological and behavioral) toxicology of TMT compounds.

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